0 (0–2), respectively. The median number of hospital admissions per patient was 1 (range 0–4). The reasons for acute care/ER visits and hospital admissions were tabulated below.

	Reasons for acute care/ER visits and hospital admissions
Acute care/ER visits	fever (n=23), bacteremia (2), dehydration (6), bronchiolitis (1), hemolysis (1), gastrointestinal bleeding (1), pseudotumor cerebri (2), bandemia (1)
Hospital admissions	conditioning (n=8), fever with no positive cultures (14), bacteremia (8), dehydration (4), bronchiolitis (1), hemolysis (1), gastrointestinal bleeding (1), pseudotumor cerebri (1), bandemia (1), CMV (1)

In 82.5% of hospital admissions (33/40), the clinical course was non-complex, with the discharge diagnosis the same as the admission diagnosis. In 17.5% (7/40), the course was complicated by conditions that developed while patients were in hospital, including bacteremia (n = 3), sepsis (3), adenovirus (1), respiratory distress (2), gastrointestinal bleeding (3), and organ failure (2). The median length of the non-complex and complex hospitalizations was 4 days (range 1–38) and 50 days (9–80), respectively. On any day, the mean probability that a patient would be an out-patient was 78.6% (CI 77.0–80.1%); the probability that the patient would be an in-patient for a non-complex or complex stay was 8.8% and 12.6%, respectively (CI 7.3–10.8% and 11.6–13.6%). In an earlier cohort, in which the busulfan AUC target was 4000–4600 microM-min (n = 17), 4 patients had primary graft failure and 3 died from related complications. In a later cohort with an AUC target of 5000 (n = 9), no patients had primary graft failure or death. Survival in these 2 cohorts was 81.2 and 100%, respectively (median follow-up 32.2 and 10.5 months). All engrafted patients have stable donor chimerism, improved lymphocyte counts, normalized immune function and decreased susceptibility to infection. Our data showed that ambulatory HSCT utilizing a RIC regimen was highly effective in children with PID.

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IMPACT OF REDUCED INTENSITY CONDITIONING (RIC) IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): A REPORT FROM THE CIBMTR

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Significant experience now exists with reduced intensity condition (RIC) regimens prior to allogeneic hematopoietic cell transplantation (HCT) in adults with acute myeloid leukemia and non-ALL, lymphoid malignancies. Due to concerns that ALL may require more intensive therapy, there are few reports on the use of RIC, particularly in children with ALL. Therefore, we evaluated the transplant outcomes after RIC and allogeneic HCT in 41 children with ALL. In this retrospective analysis, all patients were aged 0–18 years with 64% aged 11–18 years; 44% had a Karnofsky score of <90%; and the disease status at the time of transplant was: 1st complete remission (CR) in 13%, 2nd CR in 41%, and >2nd CR in 26% and 21% had active disease. The time from diagnosis to transplant for 1st CR patients was 9 months (8–62 months) and for ≥2nd CR patients it was 32 months after 1st CR (range, 6–89 months). 63% were transplanted after 2000. A TBI-based conditioning regimen was used in 33% of patients, while the remainder (66%) received non-TBI containing regimens consisting of: busulfan (34%), cyclophosphamide (15%) and melphalan (17%). Matched related donors, were available for 37% (50% BM and 50% PBSC), with the remaining (63%) having unrelated donors (BM in 19%, PBSC in 55% and cord blood in 23%). Most had GVHD prophylaxis with a calcineurin inhibitor in combination with either methotrexate (47%) or other agents (41%). The disease free survival (DFS) and overall survival (OS) at 3 yrs was 31% (95% CI, 16–47%) and 38% (95% CI, 22–54%), respectively. Transplant related mortality (TRM) at 100 days and 3 years was 18% (95% confidence interval (CI, 7–30%) and 30% (95% CI, 16–46%). The incidence of aGVHD (Grade II-IV) at day +100 was 35%. At 3 years, cGHVD developed in 23% of patients. Relapse at 3 years was 39% (95% CI, 23–57). This is the largest series describing outcomes in pediatric patients with ALL undergoing allogeneic HCT after a RIC. While additional analyses are required, these data demonstrate that long term DFS and OS can be achieved using RIC regimens in pediatric patients with ALL.

SOLID TUMORS

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ADOPTIVE TRANSFER OF HER2-SPECIFIC T CELLS ERADICATES EXPERIMENTAL GLIOBLASTOMA MULTIFORME

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Background: The intent of this project is to develop an effective adoptive immunotherapy for glioblastoma multiforme (GBM), which remains largely incurable with current treatment disciplines. New therapies are thus needed to improve current outcomes without increasing treatment-related morbidities. Immunotherapies have the potential to fulfill this need, since they are highly tumor-specific and cause minimal bystander cell damage. We propose to use T cells to target the human epidermal growth factor receptor 2 (HER2), a surface antigen, which is overexpressed in GBM. While the use of HER2 monoclonal antibodies has been limited by low levels of HER2 expression on GBMs, we show here that T cells expressing HER2-specific chimeric antigen receptors (CAR) have potent anti-tumor activity both ex vivo and in animal models.

Methods: T cells from GBM patients were retroviraly transduced to express HER2-specific CAR with a CD28.ζ signaling domain (HER2-specific T cells). Primary GBM cells and GBM cell lines were used to test the function of the generated HER2-specific T cells. The ex vivo efficacy was determined by their ability 1) to kill HER2-positive target cells in a cytoxicity assay and 2) to proliferate and secrete cytokines (IFN-γ and IL-2) in response to stimulation with HER2-positive tumor cells. The in vivo efficacy of the HER2-specific T cells was tested for the ability to induce tumor regression in an orthotopic murine xenograft model.

Results: Primary HER2-specific T cells killed both HER2-positive autologous GBM cells and GBM cell lines in cytotoxicity assays, whereas HER2-negative targets were not killed. Stimulation of HER2-specific T cells with HER2-positive primary GBM cells and GBM cell lines resulted in T-cell proliferation and secretion of IFN-γ and IL-2 in a HER2-dependent manner. Intra-tumoral injection of HER2-specific T cells resulted in eradication of established GBM xenografts in an orthotopic murine model. In contrast, delivery of non-transduced T cells did not change the tumor growth pattern.

Conclusion: We demonstrate that T-cells expressing HER2-specific CARs can recognize and kill HER2-positive GBMs. Their activation results in proliferation and secretion of immunostiumlatory cytokines. HER2-specific T cells can effectively eradicate established GBM xenografts in an orthotopic murine model. These results indicate that HER2-specific T cells could represent a promising immunotherapeutic approach for GBM.

STEM CELL BIOLOGY

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IDENTIFICATION AND ISOLATION OF THE HEMATOPOIETIC STEM CELL NICHE INITIATING CELL POPULATION

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Introduction: Identification and understanding of the cells and processes that can generate, sustain and influence the HSC niche and

hematopoiesis are critical for the development of a more comprehensive knowledge of normal hematopoiesis, stem cell homing, trafficking, differentiation and hematopoietic pathology. Growth and renewal in many tissues are initiated by stem cells, supported by the microenvironment (niche) in which they reside. While recent work has begun to describe functional interactions between stem cells and their niches, little is known about the formation of stem cell niches.

Methods & Results: We established a functional, in vivo assay (via implantation of cells under the renal capsule) to isolate the determinants of hematopoietic stem cell (HSC) niche formation and activity. Using this novel assay, we show that a population of progenitor cells (CD45-Tie2-aV+CD105+Thy1.1-; CD105+Thy1-) sorted from 15.5 dpc fetal limbs and transplanted under the adult mouse renal capsule recruit host-derived vasculatures in a VEGF dependent manner, produce donor-derived ectopic bones through endochondral ossification, and generate a marrow cavity populated by host-derived long term reconstituting HSC (LT-HSC). In contrast, CD45-Tie2-aV+ CD105+Thy1a+ (CD105+Thy1+) progenitors form bone that does not contain a marrow cavity. While analyzing these and other sorted populations, we did not observe any instances where niche was present without bone, suggesting that skeletal progenitors are necessary for initiating an HSC niche but osteoblasts alone cannot initiate and support niche activity. Suppression of factors important for HSC maintenance, such as steel factor (SLF), in progenitor populations prior to transplant did not alter their ability to initiate and support an HSC niche. On the other hand, suppression of factors involved in endochondral ossification, such as osterix and VEGF, inhibited niche generation. Furthermore, CD105+Thy1- progenitor populations derived from regions of the fetal mandible or calvaria that do not undergo endochondral ossification form only bone without marrow in our assay.

Conclusions: In addition to identifying the limb-derived skeletal progenitor capable of endochondral ossification involved and the basic mechanisms of HSC niche initiation, our study provides a functional framework by which future studies on HSC-niche interactions at the cellular level can be carried out.

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DEPLETION OF $CD3^+$ AND/OR $CD14^+$ CELLS FROM CORD BLOOD IMPROVES EX VIVO EXPANSION

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Cord blood (CB) is a viable alternative to bone marrow (BM) or mobilized peripheral blood (mPB) for transplantation. While CB is collected, typed, frozen and banked as a readily available source of ethnically-diverse tissue for transplant, a major limitation of CB transplantation is low cell dose leading to delayed engraftment and increased risk of engraftment failure. *Ex vivo* expansion of CB could improve transplant outcomes by increasing cell dose. Using a mesenchymal stem cell (MSC) co-culture system, significant levels of $CD34^+$ cell expansion can be achieved. However, the presence of non-$CD34^+$ accessory cells may impact the efficiency of expansion. Thawed CB units contain (mean ± SEM, n = 114) 1.4 ± 0.6% $CD34^+$, 39.5 ± 1.2% $CD3^+$, 17.1 ± 0.7% $CD19^+$, 15.3 ± 0.7% $CD14^+$ and 20.1 ± 1.2% $CD56^+$ cells. Preliminary experiments demonstrated that only $CD3^+$ and/or $CD14^+$ inhibited $CD34^+$ expansion. No positive or negative impact was observed for any other cell population. These data suggested $CD3^+$ and $CD14^+$ cells as candidate populations for investigation. Our hypothesis is that the depletion of $CD3^+$ and/or $CD14^+$ cells from CB MNC will markedly improve $CD34^+$ expansion in the MSC co-culture system.

Methods: $CD3^+$ and/or $CD14^+$ cells were removed from a single CB unit by magnetic depletion (MACS, Miltenyi) and the products cultured in the MSC co-culture system. Comparisons were made between the $CD34^+$ and total nucleated cell (TNC) expansion achieved in the MSC co-culture system following the incubation of MNC (nothing depleted), MNC depleted of $CD3^+$ cells (MNC-$CD3^+$), CB MNC depleted of $CD14^+$ cells (MNC-$CD14^+$) and CB MNC depleted of both $CD3^+$ and $CD14^+$ cells (MNC-($CD3^+$ + $CD14^+$)). Incubations were performed in medium containing Flt-3L, SCF, G-CSF and TPO for 7 days. On day 7, non-adherent (n/a) cells were removed and cultured in liquid culture conditions (no MSC) for a further 7 days. On Day 14, n/a cells from the co-culture flask and liquid cultures were pooled and evaluated for TNC and $CD34^+$ cell content.

Results:

Fold increase over input (range: min-max, n=3)

	TNC	$CD34^+$
MNC	3.5-19.8	11.3-12.4
MNC-$CD3^+$	21.2-47.4	19.2-51.3
MNC-$CD14^+$	8.3-27.0	23.6-32.4
MNC-($CD3^+$ + $CD14^+$)	27.8-41.0	23.9-55.1

Conclusion: The depletion of $CD3^+$ and/or $CD14^+$ cells from the CB MNC increased *ex vivo* expansion of TNC and $CD34^+$ when compared with original CB MNC expansion. GMP-compliant antibodies that target CD3 and CD14 are available (Miltenyi) and could be used for clinical evaluation of this strategy.

SUPPORTIVE CARE

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NEW MOBILIZATION STRATEGIES FOR COLLECTION OF PERIPHERAL BLOOD PROGENITOR CELLS FOR LYMPHOMA AND MYELOMA PATIENTS

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Autologus stem cell transplantation (SCT) is a common treatment modality for lymphoma and myeloma patients. The success of SCT depends on the collection of adequate number of CD34 positive cells. Standard mobilization therapy consits of granulocyte-colony-stimulating factor G-CSF and Sargramostine (GM-CSF). Some patients that are mobilized with G-CSF alone fail to collect the target cell dose while others are able to collect target dose after numerous apheresis procedures. In Autologus progenitor cell moblizaton, the addition of chemotherapy in conjunction with G-CSF/GM-CSF can substantially increase the yield of CD34 peripheral blood progenitor stem cells (PBSC), it is not without significant toxicities, additional cost and quality of life issues. Inadequate stem cell mobilization is seen in approximtely 25% of patients undergoing autotransplantation for hematologic malignancies. Experience has shown that patients who fail initial mobilization are likely to fail remobilization. Alternative strategies are needed for donors who fail to mobilize adequate number of stem cells with standard therapy. Plerixafor (AMD 3100) is a new and promising agent being studied for use in mobilization of preipheral blood progenitor cells in patients with lymphoma and myeloma. AMD 3100 is a bicyclam derivate that specifically and reversibly inhibits binding of SDF-1 to its receptor CXCR4 leading to stem cell mobilization. Plerixafor can be used alone or in conjunction with G-CSF for stem cell mobilization. Two phase III, multicenter, randomized, double-blinded, placebo-controlled studies compared AMD3100 plus G-CSF with G-CSF alone for mobilization of stem cells in patients with myeloma and NHL. Patients recieved G-CSF at 10ug/kg/day subcutaneoulsy for 4 days and on the evening of the fourth day they recieved either AMD3100 at 240 ug/kg subcutaneously or placebo. The aphereses started on day five, after the morning dose of G-CSF, and continued until $CD34^+$ was $\leq 5 \times 10^6$/kg (NHL) or $\leq 6 \times 10^6$/kg (MM) or total of four collections. Patients continued recieving their morning doses of G-CSF and evening dose of study drug until collection was complete. Patients who falied to collect $\leq 2 \times 10^6 CD34^+$ cells/kg were eligible for rescue with AMD3100 plus G-CSF. These studies confirmed that the addition of AMD3100 to G-CSF is safe and well tolerated. Patients mobilized with AMD3100 plus G-CSF were more likely to achieve a target $CD34^+$ cell count with less apheresis and had successful transplant.